Supporting Information

Total Synthesis and Stereochemistry Revision of Mannopeptimycin Aglycone

Shinichiro Fuse,^{*,†} Hirotugu Koinuma,[†] Atsushi Kimbara,[†] Miho Izumikawa,[‡] Yuto Mifune,[†]
Haiyin He,[§] Kazuo Shin-ya,^{||} Takashi Takahashi,[⊥] and Takayuki Doi^{*,◇}

[†]Department of Applied Chemistry, Tokyo Institute of Technology, Meguro-ku, Tokyo 152-8552, Japan

[‡]Japan Biological Informatics Consortium (JBIC), Koto-ku, Tokyo 135-0064, Japan

[§]Natural Products Laboratory, Worldwide Medicinal Chemistry, Pfizer Worldwide Research and Development, Groton, Connecticut 06340, United States

^{||}National Institute of Advanced Industrial Science and Technology (AIST), Koto-ku, Tokyo 135-0064, Japan

[⊥]Yokohama College of Pharmacy, Totsuka-ku, Yokohama 245-0066, Japan

[◇]Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba-ku, Sendai 980-8578, Japan

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General techniques

NMR spectra were collected using a JEOL model ECP-400, a Varian NMR System 500NB CL, or a Varian NMR System 600 NB CL in the indicated solvent. Chemical shifts are reported in units of parts per million (ppm) relative to the signal (0.00 ppm) for internal tetramethylsilane for solutions in CDCl_3 (7.26 ppm for ^1H , 77.0 ppm for ^{13}C), acetone- d_6 (7.26 ppm for ^1H , 29.8, 206.1 ppm for ^{13}C), or DMSO- d_6 (2.49 ppm for ^1H , 39.7 ppm for ^{13}C). Multiplicities are reported using the following abbreviations: s; singlet, d; doublet, dd; doublet of doublets, ddd; doublet of doublet of doublets, ddt; doublet of doublet of triplets, dt; doublet of triplets, t; triplet, q; quartet, m; multiplet, br; broad, *J*; coupling constants in Hertz (Hz). IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer. Only the strongest and/or structurally important peaks are reported as the IR data are given in cm^{-1} . Optical rotations were measured using a JASCO model P-1020 polarimeter. HRMS (ESI-TOF) were measured with a Waters LCT PremierTM XE. All reactions were monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica-gel plates (60F-254) with UV light, visualized by 10% ethanolic phosphomolybdic acid, ceric sulfate aqueous solution, or 0.5% ninyhydrin *n*-butanol solution. Dry CH_2Cl_2 , dry THF, dry toluene, and dry MeCN were obtained using a Glasscontour solvent purification system. Dry DIEA was distilled from CaH. Boc-(2*S*,3*S*)- β -MePhe-OH was purchased from NeoMPS (CA, USA). Other amino acids and coupling agents were purchased from Watanabe Chemical Industries, Ltd. (Hiroshima, Japan).

Experimental Procedures

Alcohol 7: To a solution of AcCl (10.9 mL, 154 mmol, 3.00 equiv.) in MeOH (51.2 mL) was added H-Ser(Bn)-OH (10.0 g, 51.2 mmol, 1.00 equiv.) at room temperature under argon. After being stirred at reflux temperature for 6 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in MeCN (153 mL) was added BnBr (15.2 mL, 128 mmol, 2.50 equiv.) and K_2CO_3 (35.4 g, 256 mmol, 5.00 equiv.) at room temperature under argon. After being stirred at the same temperature for 24 h, the reaction mixture was poured into H_2O and the aqueous phase was extracted with ethyl acetate. The combined extract was washed with brine, dried over MgSO_4 , and filtered, and concentrated *in vacuo*. The residue was purified by short-pad column chromatography on silica-gel (0% to 10% ethyl acetate in hexane) to give crude dibenzyl amine. The compound was used for the next reaction without further purification.

To a solution of LiAlH_4 (2.84 g, 75.0 mmol, 1.50 equiv.) in THF (40.0 mL) was slowly added a solution of the ester in THF (210 mL) at 0 °C under argon. After being stirred at the same

temperature for 1 h, the reaction mixture was diluted with Et₂O, and quenched by the addition of saturated aqueous Na₂SO₄. The resultant suspension was filtered, and the salts were washed with Et₂O. The combined organic phase was dried over MgSO₄, and filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 20% ethyl acetate in hexane) to give alcohol **7** (17.3 g, 47.8 mmol, 3 steps 93%) as a colorless oil: $[\alpha]_D^{24} +87.1$ ($c = 1.41$, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.22-7.40 (m, 15H), 4.55 (d, 1H, $J = 12.1$ Hz), 4.51 (d, 1H, $J = 12.1$ Hz), 3.87 (d, 2H, $J = 13.1$ Hz), 3.74 (dd, 1H, $J = 6.3, 9.7$ Hz), 3.60 (d, 2H, $J = 13.1$ Hz), 3.50-3.58 (m, 3H), 3.11-3.18 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 139.5, 138.2, 129.1, 128.5 $\times$ 2, 127.8, 127.6, 127.3, 73.5, 68.0, 59.8, 58.4, 54.2; FT-IR (neat) 2858, 1495, 1453, 1097, 1073, 1028, 733, 698 cm⁻¹; HRMS (ESI-TOF) calcd for C₂₄H₂₈NO₂ [M+H]⁺ 361.2042 found 362.2040.

Imine 10 and oxazolidine 12: To a solution of (COCl)₂ (1.06 mL, 12.2 mmol, 2.00 equiv.) in CH₂Cl₂ (20.0 mL) was slowly added DMSO (1.73 mL, 24.3 mmol, 4.00 equiv.) at -78 °C under argon. After being stirred at the same temperature for 15 min, the reaction mixture was slowly added to a solution of the alcohol **7** (2.20 g, 6.09 mmol, 1.00 equiv.) in CH₂Cl₂ (10.0 mL). After being stirred at the same temperature for 30 min, to the reaction mixture was added NEt₃ (5.09 mL, 36.5 mmol, 6.00 equiv.) in one portion. After being stirred at 0 °C for 30 min, the reaction mixture was poured into saturated aqueous NaHCO₃ and the aqueous phase was extracted with Et₂O. The combined extract was washed with brine, dried over MgSO₄, and filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of LiN(TMS)₂ (1.0 M in THF, 8.71 mL, 8.71 mmol, 1.43 equiv.) was slowly added a solution of N-(diphenylmethylene)glycine *tert*-butyl ester (**9**) (2.70 g, 9.14 mmol, 1.50 equiv.) in THF (80.0 mL) at -78 °C under argon. After being stirred at the same temperature for 45 min, to the reaction mixture was added a solution of the residue in THF (10.0 mL) at -78 °C. After being stirred at the same temperature for 2 h, the reaction mixture was poured into saturated aqueous NaHCO₃ and the aqueous phase was extracted with ethyl acetate. The combined extract was washed with brine, dried over Na₂SO₄, and filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 30% ethyl acetate in hexane) to give imine **10** (1.51 g, 2.31 mmol, 2 steps 38%) as a light yellow oil and oxazolidine **12** (1.39 g, 2.13 mmol, 2 steps 35%)

as a light yellow oil.

10: ^1H NMR (400 MHz, CDCl_3) δ 6.94-7.67 (m, 25H), 4.58 (ddd, 1H, $J = 5.9, 6.8, 7.7$ Hz), 4.35 (d, 2H, $J = 2.0$ Hz), 4.17 (d, 1H, $J = 7.7$ Hz), 3.85 (d, 2H, $J = 14.1$ Hz), 3.80 (d, 2H, $J = 14.1$ Hz), 3.68 (m, 1H), 3.56 (dd, 1H, $J = 4.4, 5.9$ Hz), 3.35 (d, 1H, $J = 5.9$ Hz), 3.08 (m, 1H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ 171.1, 170.7, 140.7, 139.5, 138.6, 136.4, 130.4, 129.4, 129.1 $\times$ 2, 129.0, 128.8 $\times$ 2, 128.6, 128.5, 128.4 $\times$ 2, 128.3 $\times$ 2, 128.1 $\times$ 3, 128.0 $\times$ 2, 127.5, 127.4 $\times$ 2, 127.2, 126.6 $\times$ 2, 81.9, 73.6, 73.3, 69.5, 68.9, 57.3, 55.3, 28.2; HRMS (ESI-TOF) calcd for $\text{C}_{43}\text{H}_{47}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$ 655.3536 found 655.3548. **12:** ^1H NMR (400 MHz, CDCl_3) δ 7.20-7.55 (m, 25H), 4.48 (m, 1H), 4.24 (d, 1H, $J = 11.7$ Hz), 4.13 (d, 1H, $J = 11.7$ Hz), 3.91-3.99 (m, 3H), 3.76 (d, 2H, $J = 14.1$ Hz), 3.74 (m, 1H), 3.58 (m, 1H), 3.12 (m, 1H), 3.02 (m, 1H), 1.19 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ 170.5, 144.8, 144.5, 140.6, 138.7, 130.1, 128.9 $\times$ 2, 128.6 $\times$ 2, 128.2 $\times$ 6, 127.6 $\times$ 2, 127.3 $\times$ 2, 126.8 $\times$ 2, 126.7 $\times$ 4, 126.0 $\times$ 3, 100.7, 81.9, 81.3, 72.7, 67.8, 64.1, 58.6, 55.3, 27.8; HRMS (ESI-TOF) calcd for $\text{C}_{43}\text{H}_{47}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$ 655.3536 found 655.3546.

Carbamate 13: To a solution of **10** (150 mg, 0.240 mmol, 1.00 equiv.) in Et_3SiH (0.400 mL) and H_2O (40.0 μL) was added TFA (3.60 mL) at room temperature. After being stirred at the same temperature for 3 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in THF (2.00 mL) and H_2O (2.00 mL) was added Boc_2O (67.0 mg, 0.310 mmol, 1.30 equiv.) and K_2CO_3 (98.0 mg, 0.710 mmol, 3.00 equiv.) at room temperature. After being stirred at the same temperature for 12 h, the reaction mixture was poured into 1 M HCl and the aqueous phase was extracted with CHCl_3 . The combined extract was washed with brine, dried over Na_2SO_4 , and filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in DMF (1.00 mL) was added BnBr (42.0 μL , 0.350 mmol, 1.50 equiv.) and K_2CO_3 (98.0 mg, 0.710 mmol, 3.00 equiv.) at room temperature. After being stirred at the same temperature for 12 h, the reaction mixture was poured into H_2O and the aqueous phase was extracted with ethyl acetate. The combined extract was washed with brine, dried over Na_2SO_4 , and filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 30% ethyl acetate in hexane) to give carbamate **13** (110 mg, 0.180 mmol, 3 steps 74%) as a colorless oil: $[\alpha]_{\text{D}}^{24} +41.5$ ($c = 1.05$, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.19-7.36 (m, 20H), 5.33 (d, 1H, $J = 8.8$ Hz), 4.96 (d, 1H, $J = 12.2$ Hz), 4.93 (d, 1H, $J = 12.2$ Hz), 4.69 (dd, 1H, $J = 2.2, 8.8$ Hz), 4.50 (s, 2H), 4.17 (m, 1H), 3.84 (d, 2H, $J = 13.7$ Hz), 3.77-3.84 (m, 2H), 3.56 (d, 2H, $J = 13.7$ Hz), 3.48

(d, 1H, $J = 6.3$ Hz), 3.22 (m, 1H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ 170.6, 155.9, 141.0, 139.4 $\times$ 2, 137.8, 135.5, 129.2, 128.6 $\times$ 4, 128.5 $\times$ 2, 128.4 $\times$ 2, 128.3 $\times$ 2, 127.8 $\times$ 2, 127.7, 127.5, 127.1 $\times$ 2, 127.0 $\times$ 2, 79.8, 73.8, 73.6, 67.8, 67.0, 65.2, 58.3, 57.1, 55.2, 28.4; FT-IR (neat) 3672, 2933, 1739, 1717, 1496, 1366, 1165, 699 cm^{-1} ; HRMS (ESI-TOF) calcd for $\text{C}_{38}\text{H}_{45}\text{N}_2\text{O}_6$ $[\text{M}+\text{H}]^+$ 625.3278 found 625.3295.

Oxazolidine S1: To a solution of **13** (250 mg, 0.390 mmol, 1.00 equiv.) in CH_2Cl_2 (2.00 mL) was added 2-methoxyprop-1-ene (750 μL , 20.0 mmol, 50.0 equiv.) and PPTS (50.0 mg, 0.200 mmol, 0.500 equiv.) at room temperature under argon. After being stirred at the same temperature for 12 h, the reaction mixture was quenched with NEt_3 and concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 10% ethyl acetate in hexane) to give oxazolidine **S1** (218 mg, 0.320 mmol, 82%) as a light yellow oil. The NMR spectra were observed as a mixture of rotamers. The NMR spectra of the major rotamer are shown below: $[\alpha]_D^{24} +22.5$ ($c = 1.05$, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.13-7.36 (m, 20H), 4.92 (d, 1H, $J = 12.2$ Hz), 4.79 (d, 1H, $J = 12.2$ Hz), 4.38-4.55 (m, 3H), 4.30 (d, 1H, $J = 6.4$ Hz), 3.73-3.85 (m, 5H), 3.61 (dd, 1H, $J = 2.4, 10.7$ Hz), 3.39 (m, 1H), 1.65 (s, 3H), 1.45 (s, 3H), 1.29 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ 169.7, 152.0, 140.0, 138.7, 135.4, 129.0, 128.5 $\times$ 2, 128.4 $\times$ 2, 128.3 $\times$ 2, 128.1, 127.5, 126.8, 94.5, 80.3, 76.6, 73.2, 67.4, 66.7, 62.0, 57.6, 55.5, 28.2, 25.3, 24.1; FT-IR (neat) 3437, 1748, 1707, 1637, 1376, 1255, 1185, 698 cm^{-1} ; HRMS (ESI-TOF) calcd for $\text{C}_{41}\text{H}_{49}\text{N}_2\text{O}_6$ $[\text{M}+\text{H}]^+$ 665.3591 found 665.3583.

Guanidine S2: To a solution of **S1** (350 mg, 0.526 mmol, 1.00 equiv.) in ethyl acetate (5.00 mL) was added $\text{Pd}(\text{OH})_2$ (20% on carbon, 1.05 g) at room temperature under argon. After being stirred at the same temperature for 24 h under H_2 gas atmosphere, the reaction mixture was filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in 1,4-dioxane (5.00 mL) and H_2O (250 μL) was added Goodman's reagent (363 mg, 0.790 mmol, 1.50 equiv.) and NEt_3 (0.730 mL, 5.26 mmol, 10.0 equiv.) at room temperature under argon. After being stirred at 45 $^\circ\text{C}$ for 12 h, the reaction mixture was poured into 2 M aqueous NaHSO_4 and the aqueous phase was extracted with CHCl_3 . The combined extract was washed with H_2O and brine, dried over Na_2SO_4 , and filtered, and concentrated *in vacuo*. The residue

was used for the next reaction without further purification.

To a solution of the residue in DMF (3.00 mL) was added MeI (164 μ L, 2.63 mmol, 5.00 equiv.) and K_2CO_3 (218 mg, 1.58 mmol, 3.00 equiv.) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was poured into aqueous $NaHSO_4$ and the aqueous phase was extracted with ethyl acetate. The combined extract was washed with H_2O and brine, dried over Na_2SO_4 , and filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 8% acetone in toluene) to give guanidine **S2** (224 mg, 0.337 mmol, 3 steps 64%) as a white solid. The NMR spectra were observed as a mixture of rotamers. The NMR spectra of the major rotamer are shown below: mp 49-56 $^{\circ}C$; $[\alpha]_D^{24} +23.8$ ($c = 0.87$, $CHCl_3$); 1H NMR (400 MHz, $CDCl_3$) δ 11.7 (s, 1H), 8.81 (s, 1H), 7.28-7.38 (m, 10H), 5.06-5.21 (m, 4H), 4.71 (brs, 1H), 4.49 (m, 1H), 4.40 (d, 1H, $J = 6.3$ Hz), 4.19 (m, 1H), 3.73-3.88 (m, 2H), 3.62 (s, 3H), 1.74 (s, 3H), 1.53 (s, 3H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 163.3, 156.2, 153.8, 151.0, 136.6, 134.6, 128.9, 128.8×2 , 128.6×2 , 128.5×2 , 128.3, 128.2, 128.0, 95.5, 80.7, 75.3, 68.5, 67.3, 62.2, 61.5, 52.8, 52.5, 28.4, 25.3, 24.6; FT-IR (neat) 3434, 1732, 1706, 1623, 1382, 1338, 1053, 754, 698 cm^{-1} ; HRMS (ESI-TOF) calcd for $C_{31}H_{41}N_4O_{10}$ $[M+H]^+$ 629.2823 found 629.2843.

Protected Aiha-A 15: To a solution of **S2** (30.0 mg, 47.7 μ mol, 1.00 equiv.) in CH_2Cl_2 (1.00 mL) was added NEt_3 (33.3 μ L, 239 μ mol, 5.00 equiv.) and $MsCl$ (4.10 μ L, 52.5 μ mol, 1.20 equiv.) at 0 $^{\circ}C$ under argon. After being stirred at room temperature for 5 h, the reaction mixture was poured into 2 M aqueous $NaHSO_4$ and the aqueous phase was extracted with ethyl acetate. The combined extract was washed with brine, dried over Na_2SO_4 , and filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 10% acetone in toluene) to give cyclic guanidine **15** (25.7 mg, 42.0 μ mol, 88%) as a white solid. The NMR spectra were observed as a mixture of rotamers. The NMR spectra of the major rotamer are shown below: mp 58-61 $^{\circ}C$; $[\alpha]_D^{25} +19.1$ ($c = 0.77$, $CHCl_3$); 1H NMR (400 MHz, $acetone-d_6$) δ 9.02 (brs, 1H), 7.29-7.56 (m, 10H), 5.26 (s, 2H), 5.13 (s, 2H), 4.62 (m, 1H), 4.54 (m, 1H), 4.07 (m, 1H), 3.98 (m, 1H), 3.91 (m, 1H), 3.80 (s, 3H), 1.66 (s, 3H), 1.47 (s, 3H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 151.8, 150.9, 150.4, 136.4, 135.2, 128.7, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 95.8, 95.1, 81.4, 77.7, 68.5, 67.5, 61.4, 61.2, 52.8, 52.7, 28.4, 26.4, 25.5, 24.7; FT-IR (solid) 3352, 1759, 1712, 1657, 1615, 1368, 1309, 1258, 1170 cm^{-1} ; HRMS (ESI-TOF) calcd for $C_{31}H_{39}N_4O_9$ $[M+H]^+$ 611.2717 found 611.2710.

Amino alcohol 16: To a solution of **12** (800 mg, 1.22 mmol, 1.00 equiv.) in Et₃SiH (2.00 mL) and H₂O (200 μ L) was added TFA (18.0 mL) at room temperature. After being stirred at the same temperature for 3 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in THF (10.0 mL) and H₂O (10.0 mL) was added Boc₂O (347 mg, 1.59 mmol, 1.30 equiv.) and K₂CO₃ (506 mg, 3.66 mmol, 3.00 equiv.) at room temperature. After being stirred at the same temperature for 12 h, the reaction mixture was poured into 1 M HCl and the aqueous phase was extracted with CHCl₃. The combined extract was washed with brine, dried over Na₂SO₄, and filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in DMF (5.00 mL) was added MeI (0.220 mL, 1.83 mmol, 1.50 equiv.) and K₂CO₃ (506 mg, 3.66 mmol, 3.00 equiv.) at room temperature. After being stirred at the same temperature for 12 h, the reaction mixture was poured into H₂O and the aqueous phase was extracted with ethyl acetate. The extract was washed with brine, dried over Na₂SO₄, and filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 30% ethyl acetate in hexane) to give amino alcohol **16** (540 mg, 0.990 mmol, 3 steps 81%) as a colorless oil: $[\alpha]_D^{23} +13.5$ ($c = 1.05$, CHCl₃); ¹H NMR (400 MHz, acetone-*d*₆) δ 7.18-7.43 (m, 15H), 5.31 (d, 1H, $J = 9.3$ Hz), 4.92 (d, 1H, $J = 9.3$ Hz), 4.58 (d, 1H, $J = 12.2$ Hz), 4.54 (d, 1H, $J = 12.2$ Hz), 4.44 (brs, 1H), 4.43 (brs, 1H), 3.95 (dd, 1H, $J = 5.4, 5.8$ Hz), 3.94 (d, 2H, $J = 13.7$ Hz), 3.88 (dd, 1H, $J = 5.4, 10.3$ Hz), 3.67 (d, 2H, $J = 13.7$ Hz), 3.63 (s, 3H), 2.96 (m, 1H), 1.44 (s, 9H); ¹³C NMR (100 MHz, acetone-*d*₆) δ 173.6, 156.8, 140.6 $\times 2$, 139.6, 129.7 $\times 4$, 129.0 $\times 6$, 128.4 $\times 2$, 128.2, 127.5 $\times 2$, 79.2, 73.8, 70.9, 67.9, 59.3, 56.1, 56.0, 55.9, 52.0, 28.6; FT-IR (neat) 3420, 2960, 1720, 1706, 1505, 1338, 1160, 685 cm⁻¹; HRMS (ESI-TOF) calcd for C₃₂H₄₁N₂O₆ [M+H]⁺ 549.2964 found 549.2849.

Oxazolidine S3: To a solution of **16** (800 mg, 1.27 mmol, 1.00 equiv.) in CH₂Cl₂ (10.0 mL) was added 2-methoxyprop-1-ene (2.38 mL, 25.4 mmol, 20.0 equiv.) and PPTS (105 mg, 0.410 mmol, 0.300 equiv.) at room temperature under argon. After being stirred at the same temperature for 12 h, the reaction mixture was quenched with NEt₃ and concentrated *in vacuo*. The residue was purified

by column chromatography on silica-gel (0% to 10% ethyl acetate in hexane) to give oxazolidine **S3** (765 mg, 1.12 mmol, 88%) as a light yellow oil. The NMR spectra were observed as a mixture of rotamers. The NMR spectra of the major rotamer are shown below: $[\alpha]_D^{24} +5.49$ ($c = 1.24$, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.18-7.38 (m, 15H), 4.56 (s, 2H), 4.44 (d, 1H, $J = 4.9$ Hz), 4.36 (dd, 1H, $J = 5.2, 5.8$ Hz), 3.89 (d, 1H, $J = 4.4$ Hz), 3.85 (d, 1H, $J = 5.8$ Hz), 3.81 (d, 2H, $J = 13.1$ Hz), 3.65 (d, 2H, $J = 13.7$ Hz), 3.64 (s, 3H), 3.08 (ddd, 1H, $J = 5.8, 5.8, 5.8$ Hz), 1.58 (s, 3H), 1.45 (s, 3H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ 172.2, 151.3, 139.9×2 , 138.6, 129.1×3 , 128.4×4 , 128.3×2 , 127.6×4 , 127.0×2 , 96.0, 80.2, 78.4, 73.5, 67.1, 62.3, 58.0, 55.1, 52.1, 28.3, 27.1, 25.0; FT-IR (neat) 3434, 2980, 1756, 1710, 1378, 1247, 1202, 699 cm^{-1} ; HRMS (ESI-TOF) calcd for $\text{C}_{35}\text{H}_{45}\text{N}_2\text{O}_6$ $[\text{M}+\text{H}]^+$ 589.3278 found 589.3263.

Protected Aiha-B 18: To a solution of **S3** (220 mg, 0.370 mmol, 1.00 equiv.) in MeOH (22.0 mL) was added Pd (10% on carbon, 220 mg), HCO_2H (143 μL , 3.74 mmol, 10.0 equiv.) and NEt_3 (261 μL , 1.87 mmol, 5.00 equiv.) at room temperature under argon. After being stirred at reflux temperature for 4 h, the reaction mixture was filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in 1,4-dioxane (3.70 mL) and H_2O (190 μL) was added Goodman's reagent (170 mg, 0.370 mmol, 1.00 equiv.) and NEt_3 (261 μL , 1.87 mmol, 5.00 equiv.) at room temperature under argon. After being stirred at 45 $^\circ\text{C}$ for 24 h, the reaction mixture was poured into 2 M aqueous NaHSO_4 and the aqueous phase was extracted with ethyl acetate. The extract was washed with saturated aqueous NaHCO_3 and brine, dried over Na_2SO_4 , and filtered and concentrated *in vacuo*. The residue was purified by short-pad column chromatography on silica-gel (0% to 8% acetone in toluene) to give crude guanidine. The compound was used for the next reaction without further purification.

To a solution of the residue in CH_2Cl_2 (6.40 mL) was added NEt_3 (258 μL , 1.85 mmol, 5.00 equiv.) and MsCl (31.8 μL , 0.407 mmol, 1.10 equiv.) at 0 $^\circ\text{C}$ under argon. After being stirred at room temperature for 12 h, the reaction mixture was poured into H_2O and the aqueous phase was extracted with ethyl acetate. The combined extract was washed with brine, dried over Na_2SO_4 , and filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 12% acetone in toluene) to give **18** (180 mg, 0.300 mmol, 3 steps 80%) as a white solid. The NMR spectra were observed as a mixture of rotamers. The NMR spectra of the major rotamer are shown below: mp 60-64 $^\circ\text{C}$; $[\alpha]_D^{24} +16.4$ ($c = 1.00$, CHCl_3); ^1H NMR (400 MHz, $\text{acetone-}d_6$) δ 8.93 (brs, 1H), 7.31-7.55 (m, 10H), 5.26 (s, 2H), 5.12 (s, 2H), 4.33 (m, 1H), 4.32 (m, 1H), 4.29 (m, 1H), 4.12

(dd, 1H, $J = 9.9, 10.3$ Hz), 3.95 (m, 1H), 3.73 (s, 3H), 1.57 (s, 3H), 1.54 (s, 3H), 1.36 (s, 9H); ^{13}C NMR (100 MHz, acetone- d_6) δ 171.7, 158.1, 151.7, 151.5 $\times$ 2, 138.4, 137.0, 129.3 $\times$ 2, 129.2 $\times$ 2, 129.1 $\times$ 2, 128.7 $\times$ 2, 128.5 $\times$ 2, 96.4, 80.8, 68.3, 67.5, 62.4, 52.8, 46.4, 28.2, 26.6, 24.3; FT-IR (neat) 3352, 1759, 1712, 1657, 1615, 1368, 1309, 1258, 1169 cm^{-1} ; HRMS (ESI-TOF) calcd for $\text{C}_{31}\text{H}_{39}\text{N}_4\text{O}_9$ $[\text{M}+\text{H}]^+$ 611.2717 found 611.2711.

Structural confirmation of Aiha-A S4 (TFA salt): To a solution of **15** (4.5 mg, 7.5 μmol , 1.0 equiv.) in MeOH (0.300 mL) was added Pd/C (10% on carbon, 9.0 mg) at room temperature under argon. After being stirred at the same temperature for 2 h under H_2 gas atmosphere, the reaction mixture was filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in CH_2Cl_2 (1.40 mL) was added Et_3SiH (30.0 μL) and TFA (0.750 mL) at room temperature. After being stirred at the same temperature for 2 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in 1,4-dioxane (1.20 mL) and H_2O (1.20 mL) was added NaHCO_3 (1.9 mg, 22 μmol , 3.0 equiv.) and Cbz-OSu (Su, succinimide) (2.1 mg, 8.5 μmol , 1.1 equiv.) at 0 $^\circ\text{C}$ under argon. After being stirred at room temperature for 12 h, the reaction mixture was acidified with 5% TFA in MeOH and concentrated *in vacuo*. The residue was purified by reversed phase column chromatography (Bond Elut-C18: 0% to 50% MeOH in H_2O) to give Aiha-A **S4** (TFA salt) (1.7 mg, 5.0 μmol , 3 steps 67%) as a white solid: mp 43-46 $^\circ\text{C}$; ^1H NMR (400 MHz, CD_3OD) δ 7.25-7.40 (m, 5H), 5.10 (s, 2H), 4.32 (d, 1H, $J = 6.0$ Hz), 4.15 (ddd, 1H, $J = 5.8, 5.8, 9.0$ Hz), 3.87 (dd, 1H, $J = 5.8, 5.8$ Hz), 3.75 (s, 3H), 3.70 (m, 2H); HRMS (ESI-TOF) calcd for $\text{C}_{15}\text{H}_{21}\text{N}_4\text{O}_5$ $[\text{M}+\text{H}]^+$ 337.1512 found 337.1516. The observed ^1H NMR spectrum was well consisted to that of the reported spectrum.¹

Structural confirmation of Aiha-B S5 (TFA salt): To a solution of **18** (9.0 mg, 15 μmol , 1.0 equiv.) in MeOH (0.600 mL) was added Pd/C (10% on carbon, 18.0 mg) at room temperature under argon. After being stirred at the same temperature for 2 h under H_2 gas atmosphere, the reaction mixture was filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in CH₂Cl₂ (2.80 mL) was added Et₃SiH (60.0 μL) and TFA (0.750 mL) at room temperature. After being stirred at the same temperature for 2 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in 1,4-dioxane (1.50 mL) and H₂O (1.50 mL) was added NaHCO₃ (3.8 mg, 45 μmol, 3.0 equiv.) and Cbz-OSu (3.9 mg, 16 μmol, 1.1 equiv.) at 0 °C under argon. After being stirred at room temperature for 12 h, the reaction mixture was acidified with 5% TFA in MeOH and concentrated *in vacuo*. The residue was purified by reversed phase column chromatography (Bond Elut-C18: 0% to 50% MeOH in H₂O) to give Aiha-B **S5** (TFA salt) (3.6 mg, 5.1 μmol, 3 steps 70%) as a white solid: ¹H NMR (400 MHz, CD₃OD) δ 7.30-7.40 (m, 5H), 5.15 (s, 2H), 4.52 (d, 1H, *J* = 1.5 Hz), 4.08 (dd, 1H, *J* = 1.5, 7.7 Hz), 3.86 (ddd, 1H, *J* = 7.7, 7.7, 7.7 Hz), 3.78 (s, 3H), 3.69 (d, 2H, *J* = 7.7 Hz); HRMS (ESI-TOF) calcd for C₁₅H₂₁N₄O₅ [M+H]⁺ 337.1512 found 337.1519. The observed ¹H NMR spectrum was well consisted to that of the reported spectrum.¹

Amino alcohol S7: To **15** (110 mg, 175 μ mol, 1.00 equiv.) was added a solution of 4 M HCl in 1,4-dioxane (2.00 mL) and H₂O (20 μ L) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in CH₂Cl₂ (2.00 mL) was added Boc₂O (220 mg, 0.875 mmol, 5.00 equiv.) and K₂CO₃ (72.6 mg, 0.525 mmol, 3.00 equiv.) at room temperature. After being stirred at the same temperature for 12 h, the reaction mixture was poured into 2 M aqueous NaHSO₄ and the aqueous phase was extracted with ethyl acetate. The combined extract was washed with brine, dried over Na₂SO₄, and filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 20% acetone in toluene) to give amino alcohol **S7** (103 mg, 168 μ mol, 2 steps 96%) as a white solid: $[\alpha]_D^{24} +21.7$ ($c = 1.00$, CHCl₃); ¹H NMR (400 MHz, acetone-*d*₆) δ 8.82 (brs, 1H), 7.28-7.56 (m, 10H), 6.28 (d, 1H, $J = 7.7$ Hz), 5.26 (s, 2H), 5.11 (s, 2H), 4.34 (m, 1H), 4.16 (m, 1H), 4.09 (m, 1H), 4.02 (m, 2H), 3.70 (s, 3H), 1.43 (s, 9H); ¹³C NMR (100 MHz, acetone-*d*₆) δ 171.3, 156.1, 151.7 $\times$ 3, 138.1, 136.8, 129.0 $\times$ 2, 128.9 $\times$ 2, 128.6 $\times$ 3, 128.4 $\times$ 2, 128.3, 79.8, 79.0, 73.4, 68.1, 67.4, 56.9, 52.2, 46.1, 28.3; FT-IR (neat) 3650, 1571, 1488, 1457, 897, 868, 840, 641 cm⁻¹; HRMS (ESI-TOF) calcd for C₂₈H₃₅N₄O₉ [M+H]⁺ 571.2404 found 571.2413.

Boc-Aiha-A-Aiha-B-Ser(Bn)-OAllyl 22: To a solution of Boc-Aiha-B-Ser(Bn)-OAllyl **S6** (11.6 mg, 14.3 μ mol, 1.1 equiv.) was added a solution of 4 M HCl in 1,4-dioxane (1.5 mL) and H₂O (15 μ L) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue of **20** was used for the next reaction without further purification.

To a solution of **S7** (7.4 mg, 13 μ mol, 1.0 equiv.) in THF (535 μ L) and H₂O (107 μ L) was added LiOH·H₂O (3.0 mg, 64 μ mmol, 4.9 equiv.) at 0 °C. After being stirred at the same temperature for 10 min, the reaction mixture was poured into ice-cooled 1 M HCl and the aqueous phase was extracted with CHCl₃. The combined extract was dried over Na₂SO₄, and filtered and concentrated *in vacuo*. The residue of the carboxylic acid **21** was used for the next reaction without further

purification.

To a solution of the carboxylic acid **21** residue in CH₂Cl₂ (0.300 mL) was added the residue amine **20**, DIEA (7.2 μ L, 39 μ mol, 3.0 equiv.), and PyBOP (7.5 mg, 20 μ mol, 1.5 equiv.) at room temperature under argon. After being stirred at the same temperature for 12 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by chromatography on silica-gel (0% to 5% MeOH in CHCl₃) to give Boc-Aiha-A-Aiha-B-Ser(Bn)-OAllyl **22** (11.9 mg, 9.82 μ mol, 2 steps 75%) as a colorless oil: HRMS (ESI-TOF) calcd for C₆₂H₇₀N₉O₁₇ [M+H]⁺ 1212.4890 found 1212.4893. The solubility of the obtained tripeptide **22** against various solvents was insufficient for NMR analysis. Therefore, protecting groups were removed for structural confirmation of the tripeptide, as shown below.

HPLC analysis: Inertsil® ODS-3 column: 4.6 $\times$ 250 mm, 3 μ m, linear gradient solvent: 10-100% MeCN/H₂O with 0.1% formic acid over 15 min, flow rate: 1.0 mL/min. The retention time of **22**: 9.13 min.

Boc-Aiha-A-Aiha-B-Ser-OH (S8) (TFA salt): To a solution of Boc-Aiha-A-Aiha-B-Ser(Bn)-OAllyl **22** (10.0 mg, 8.26 μ mol, 1.00 equiv.) was added Pd(PPh₃)₄ (9.6 mg, 8.3 μ mol, 100 mol%) and DMBA (1.3 mg, 8.3 μ mol, 1.0 equiv.) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by chromatography on silica-gel (0% to 10% MeOH in CHCl₃).

To a solution of the residue in MeOH (0.500 mL), THF (0.500 mL), H₂O (0.125 mL) and HCO₂H (65.0 μ L) was added Pd(OH)₂ (20% on carbon, 20.0 mg) at room temperature under argon. After being stirred at the same temperature for 8 h under H₂ gas atmosphere, the reaction mixture was filtered and concentrated *in vacuo*. The residue was purified by reversed phase column chromatography (Bond Elut-C18: 5% to 40% MeCN in H₂O, 0.02% TFA) to give Boc-Aiha-A-Aiha-B-Ser-OH (**S8**) (TFA salt) (3.6 mg, 6.7 μ mol, 2 steps 81%) as a white solid: [α]_D²⁴ +7.93 (c = 0.30, H₂O/MeOH = 1/1); ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.27 (brd, 1H, *J* = 7.8 Hz), 8.09 (brs, 1H), 8.02 (brs, 1H), 7.98 (brd, 1H, *J* = 7.8 Hz), 7.93 (brs, 1H), 7.60 (brs, 1H), 7.14 (m, 1H), 5.73 (brs, 1H), 5.64 (brs, 1H), 4.55 (brd, 1H, *J* = 7.8 Hz), 4.30 (m, 1H), 4.11 (m, 1H), 3.96 (m, 2H), 3.79 (m, 1H), 3.72 (m, 1H), 3.69 (m, 1H), 3.63 (m, 2H), 3.53 (m, 3H), 1.37 (s, 9H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 171.9, 170.6, 169.1, 159.5, 159.3, 155.4, 79.0, 71.7, 71.4, 61.5, 56.4 $\times$ 2, 56.2, 55.1, 54.3, 44.9, 43.7; HRMS (ESI-TOF) calcd for C₂₀H₃₆N₉O₉ [M+H]⁺ 546.2636 found 546.2640.

Boc-(2*S*,3*S*)-β-MePhe-D-Tyr(Bn)-OMe (S9): To a solution of Boc-(2*S*,3*S*)-β-MePhe-OH (150 mg, 0.537 mmol, 1.00 equiv.) in CH₂Cl₂ (5.40 mL) was added H-D-Tyr(Bn)-OMe (HCl salt) (190 mg, 0.591 mmol, 1.10 equiv.), DIEA (0.275 mL, 1.60 mmol, 3.00 equiv.), and PyBOP (363 mg, 0.699 mmol, 1.30 equiv.) at room temperature. After being stirred at the same temperature for 12 h, the reaction mixture was poured into 3 M HCl and the aqueous phase was extracted with ethyl acetate. The combined extract was washed with H₂O and aqueous NaHCO₃ and brine, dried over Na₂SO₄, and filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 40% ethyl acetate in hexane) to give **S9** (272 mg, 0.499 mmol, 2 steps 93%) as a white solid: mp 112-116 °C; $[\alpha]_D^{22}$ -3.10 (*c* = 1.19, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.16-7.42 (m, 10H), 6.96 (d, 2H, *J* = 8.7 Hz), 6.87 (d, 2H, *J* = 8.7 Hz), 6.18 (d, 1H, *J* = 6.7 Hz), 5.02 (s, 2H), 4.87 (d, 1H, *J* = 6.2 Hz), 4.77 (ddd, 1H, *J* = 6.3, 6.3, 6.7 Hz), 4.28 (m, 1H), 3.64 (s, 3H), 3.45 (m, 1H), 2.92-3.01 (m, 2H), 1.39 (s, 9H), 1.25 (d, 3H, *J* = 7.2 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 171.6, 170.6, 158.0, 155.5, 141.4, 137.0, 130.2, 128.6 × 2, 128.0, 127.9, 127.5 × 2, 127.0, 115.1, 80.0, 70.0, 60.0, 53.3, 52.2, 40.9, 37.2, 28.3, 17.0; FT-IR (neat) 3683, 3421, 3019, 2400, 1689, 1511, 1217, 760 cm⁻¹; HRMS (ESI-TOF) calcd for C₃₂H₃₉N₂O₆ [M+H]⁺ 547.2808 found 547.2816.

Alloc-Gly-(2*S*,3*S*)-β-MePhe-D-Tyr(Bn)-OMe (23a): To a solution of **S9** (110 mg, 192 μmol, 1.00 equiv.) was added a solution of 4 M HCl in 1,4-dioxane (4.00 mL) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in CH₂Cl₂ (2.00 mL) was added Alloc-Gly-OH (36.6 mg, 0.230 mmol, 1.20 equiv.), DIEA (99.3 μL, 0.576 mmol, 3.00 equiv.), and PyBOP (150 mg, 0.288 mmol, 1.50 equiv.) at room temperature. After being stirred at the same temperature for 12 h, the reaction mixture was poured into 3 M HCl and the aqueous phase was extracted with ethyl acetate. The combined extract was washed with H₂O, saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, and filtered and concentrated *in vacuo*. The residue was purified by column chromatography on

silica-gel (0% to 40% ethyl acetate in hexane) to give **23a** (111 mg, 175 μ mol, 2 steps 91%) as a white solid: $[\alpha]_D^{22}$ -6.18 (c = 1.00, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.11-7.42 (m, 10H), 6.99 (d, 2H, J = 8.7 Hz), 6.89 (d, 2H, J = 8.7 Hz), 6.43 (d, 1H, J = 8.2 Hz), 6.37 (d, 1H, J = 7.3 Hz), 5.88 (m, 1H), 5.20-5.31 (m, 3H), 5.03 (s, 2H), 4.75 (ddd, 1H, J = 6.8, 6.8, 7.3 Hz), 4.58 (dd, 1H, J = 6.3, 8.2 Hz), 4.53 (d, 2H, J = 5.3 Hz), 3.64-3.77 (m, 5H), 3.39 (dd, 1H, J = 6.3, 6.8 Hz), 2.91-3.02 (m, 2H), 1.23 (d, 3H, J = 6.8 Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 171.7, 169.9, 169.0, 158.1, 156.4, 141.2, 137.0, 132.5, 130.3, 128.8, 128.7, 128.1, 128.0, 127.8, 127.5, 127.3, 118.1, 115.1, 70.1, 66.2, 58.6, 53.5, 52.4, 44.5, 40.9, 37.2, 17.1; FT-IR (neat) 3411, 3300, 3017, 2400, 1735, 1654, 1510, 1214, 752 cm^{-1} ; HRMS (ESI-TOF) calcd for $\text{C}_{33}\text{H}_{38}\text{N}_3\text{O}_7$ $[\text{M}+\text{H}]^+$ 588.2710 found 588.2710.

Boc-Gly-(2S,3S)- β -MePhe-D-Tyr(Bn)-OMe (23b): To a solution of **23a** (30.0 mg, 47.5 μ mol, 1.00 equiv.) in 1,4-dioxane/ H_2O = 3/1 (1.00 mL) was added $\text{Pd(PPh}_3)_4$ (3.0 mg, 2.6 μ mol, 5.5 mol%) and DMBA (7.4 mg, 48 μ mol, 1.0 equiv.) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in THF (1.40 mL) and H_2O (0.700 mL) was added Boc_2O (51.2 mg, 0.238 mmol, 5.00 equiv.) and K_2CO_3 (19.6 mg, 0.142 mmol, 3.00 equiv.) at room temperature. After being stirred at the same temperature for 12 h, the reaction mixture was poured into 2 M aqueous NaHSO_4 and the aqueous phase was extracted with ethyl acetate. The combined extract was washed with saturated aqueous NaHCO_3 and brine, dried over Na_2SO_4 , and filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0 % to 40 % ethyl acetate in hexane) to give **23b** (24.1 mg, 39.0 μ mol, 2 steps 82%) as a white solid: mp 93-97 $^\circ\text{C}$; $[\alpha]_D^{23}$ -7.12 (c = 0.935, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.11-7.42 (m, 10H), 6.99 (d, 2H, J = 8.7 Hz), 6.88 (d, 2H, J = 8.7 Hz), 6.54 (d, 1H, J = 8.2 Hz), 6.52 (d, 1H, J = 7.7 Hz), 5.03 (m, 1H), 5.02 (s, 2H), 4.73 (ddd, 1H, J = 6.8, 6.8, 7.7 Hz), 4.62 (dd, 1H, J = 6.3, 8.2 Hz), 3.62-3.70 (m, 5H), 3.40 (dd, 1H, J = 6.3, 7.2 Hz), 2.92-3.02 (m, 2H), 1.41 (s, 9H), 1.22 (d, 3H, J = 7.2 Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 171.7, 170.0, 169.4, 158.0, 141.2, 137.0, 130.3, 129.1, 128.7, 128.6, 128.2, 128.0, 127.8, 127.5, 127.2, 125.4, 115.1, 80.4, 70.1, 58.4, 53.6, 52.3, 44.4, 40.9, 37.1, 21.5, 17.0; FT-IR (neat) 3291, 3017, 1743, 1649, 1511, 1367, 1245, 1175, 1026, 747 cm^{-1} ; HRMS (ESI-TOF) calcd for $\text{C}_{34}\text{H}_{42}\text{N}_3\text{O}_7$ $[\text{M}+\text{H}]^+$ 604.3022 found 604.3024.

Boc-Gly-(2*S*,3*S*)-β-MePhe-D-Tyr(Bn)-Aiha-A-Aiha-B-Ser(Bn)-OAllyl **26:** To a solution of **22** (9.0 mg, 7.4 μmol, 1.0 equiv.) was added a solution of 4 M HCl in 1,4-dioxane (2.00 mL) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue of **S10** was used for the next reaction without further purification.

To a solution of Boc-Gly-(2*S*,3*S*)-β-MePhe-D-Tyr(Bn)-OMe (**23b**) (7.1 mg, 11 μmol, 1.5 equiv.) in THF (300 μL) and H₂O (60 μL) was added LiOH·H₂O (1.7 mg, 36 μmol, 4.9 equiv.) at room temperature. After being stirred at the same temperature for 2 h, the reaction mixture was poured into ice-cooled 1 M HCl and the aqueous phase was extracted with CHCl₃. The combined extract was dried over Na₂SO₄, and filtered and concentrated *in vacuo*. The residue of **S11** was used for the next reaction without further purification.

To a solution of the residue of **S10** in CH₂Cl₂ (0.250 mL) was added the residue of **S11**, DIEA (5.1 μL, 30 μmol, 4.0 equiv.), and HATU (8.4 mg, 22 μmol, 3.0 equiv.) at room temperature under argon. After being stirred at the same temperature for 12 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 4% MeOH in CHCl₃) to give **26** (10 mg, 5.9 μmol, 2 steps 80%) as a white solid: HRMS (ESI-TOF) C₉₀H₉₉N₁₂O₂₁ [M+H]⁺ calcd for 1683.7048 found 1683.7085. The solubility of the obtained hexapeptide **26** against various solvents was insufficient for NMR analysis. Therefore, protecting groups were removed for structural confirmation of the hexapeptide as shown below.

Boc-Gly-(2*S*,3*S*)- β -MePhe-D-Tyr-Aiha-A-Aiha-B-Ser-OH (S12) (TFA salt): To a solution of **26** (4.0 mg, 2.4 μ mol, 1.0 equiv.) in 1,4-dioxane/H₂O = 3/1 (1.00 mL) was added Pd(PPh₃)₄ (0.4 mg, 0.4 μ mol, 15 mol%) and DMBA (0.4 mg, 2.4 μ mol, 1 equiv.) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue of **27** was used for the next reaction without further purification.

To a solution of the residue of **27** in MeOH (0.500 mL), THF (0.500 mL), H₂O (125 μ L) and HCO₂H (62.5 μ L) was added Pd(OH)₂ (20% on carbon, 8.0 mg) at room temperature under argon. After being stirred at the same temperature for 24 h under H₂ gas atmosphere, the reaction mixture was filtered and concentrated *in vacuo*. The residue was purified by reversed phase column chromatography (Bond Elut-C18: 0% to 40% MeOH in H₂O, 0.02% TFA) to give **S12** (TFA salt) (2.0 mg, 1.9 μ mol, 2 steps 80%) as a white solid. The NMR spectra were observed as a mixture of rotamers. The NMR spectra of the major rotamer are shown below: $[\alpha]_D^{23} +37.8$ (c = 0.09, H₂O/MeOH = 1/1); ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.20 (brs, 1H), 8.66 (brs, 1H), 8.41 (brs, 1H), 8.22 (brs, 1H), 8.18 (brs, 1H), 7.60-8.10 (m, 6H), 7.32 (brd, 1H, *J* = 6.6 Hz), 7.13 (d, 2H, *J* = 8.4 Hz), 7.11 (m, 3H), 6.92 (m, 1H), 6.82 (m, 2H), 6.66 (m, 2H), 5.69 (brs, 2H), 4.57 (m, 1H), 4.54 (m, 1H), 4.52 (m, 1H), 4.41 (m, 1H), 4.24 (m, 1H), 3.91 (m, 1H), 3.85 (m, 1H), 3.67 (m, 3H), 3.60 (m, 2H), 3.53 (m, 2H), 3.48 (m, 1H), 3.39 (m, 1H), 3.37 (m, 1H), 3.34 (m, 1H), 2.89 (m, 1H), 2.84 (m, 1H), 2.67 (m, 1H), 1.34 (s, 9H), 0.88 (brs, 3H); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 172.3, 171.8, 170.7, 170.3, 169.4, 169.1, 159.5, 159.3, 156.2, 155.9, 142.4, 130.5, 128.1, 127.9, 127.8, 126.4, 115.1, 78.4, 72.0, 70.8, 61.7, 57.1, 56.3 $\times$ 2, 55.0, 54.7, 55.4, 55.3, 44.7, 43.4, 43.2, 42.0, 37.3, 28.3, 18.5; HRMS (ESI-TOF) calcd for C₄₁H₅₉N₁₂O₁₃ [M+H]⁺ 927.4324 found 927.4351.

Macrolactam 28: To a solution of the residue of **26** (10.4 mg, 6.18 μmol , 1.00 equiv.) in 1,4-dioxane/ H_2O = 3/1 (1.00 mL) was added $\text{Pd}(\text{PPh}_3)_4$ (1.0 mg, 0.90 μmol , 15 mol%) and DMBA (1.0 mg, 6.2 μmol , 1.0 equiv.) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by short-pass column chromatography on silica-gel (0% to 20% MeOH in CHCl_3). The obtained residue was used for the next reaction without further purification.

To a solution of the residue in CH_2Cl_2 (0.500 mL) was added TFA (25.0 μL) at room temperature under argon. After being stirred at the same temperature for 30 min, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in CH_2Cl_2 (3.09 mL) was added DIEA (4.3 μL , 25 μmol , 4.0 equiv.) and HATU (11.7 mg, 30.9 μmol , 5.00 equiv.) at room temperature under argon. After being stirred at the same temperature for 24 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 5% MeOH in CHCl_3) to give **28** (6.4 mg, 4.2 μmol , 3 steps 68%) as a white solid: HRMS (ESI-TOF) calcd for $\text{C}_{82}\text{H}_{85}\text{N}_{12}\text{O}_{18}$ $[\text{M}+\text{H}]^+$ 1525.6105 found 1525.6111. The solubility of the obtained macrolactam **28** against various solvents was insufficient for NMR analysis.

Proposed mannopeptimycin aglycone (6) (TFA salt): To a solution of **28** (4.0 mg, 2.6 μmol , 1.0 equiv.) in MeOH (0.50 mL), THF (0.50 mL), H_2O (130 μL) and HCO_2H (63 μL) was added $\text{Pd}(\text{OH})_2$ (20% on carbon, 8.0 mg) at room temperature under argon. After being stirred at the same temperature for 24 h under H_2 gas atmosphere, then the reaction mixture was filtered and

concentrated *in vacuo*. The residue was purified by reversed phase column chromatography (Bond Elut-C18: 0% to 40% MeOH in H₂O, 0.02% TFA) to give **6** (TFA salt) (2.0 mg, 2.1 μ mol, 82%) as a white solid: $[\alpha]_D^{22}$ -1.7 (*c* = 0.05, MeOH/H₂O = 1:1); ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.12 (brs, 1H), 8.50 (d, 1H, *J* = 9.0 Hz), 8.49 (d, 1H, *J* = 6.0 Hz), 8.04 (brs, 1H), 8.00 (d, 1H, *J* = 7.8 Hz), 7.94 (brs, 1H), 7.82 (d, 1H, *J* = 9.0 Hz), 7.81 (m, 1H), 7.79 (t, 1H, *J* = 6.0 Hz), 7.78 (m, 1H), 7.73 (brs, 1H), 7.67 (brs, 1H), 7.63 (d, 1H, *J* = 9.1 Hz), 7.18 (dd, 2H, *J* = 7.2, 7.2 Hz), 7.12 (t, 1H, *J* = 7.2 Hz), 7.08 (d, 2H, *J* = 8.5 Hz), 6.99 (d, 2H, *J* = 7.2 Hz), 6.63 (d, 2H, *J* = 8.5 Hz), 5.58 (d, 1H, *J* = 5.6 Hz), 5.49 (d, 1H, *J* = 5.9 Hz), 4.99 (t, 1H, *J* = 5.3 Hz), 4.55 (m, 1H), 4.54 (m, 1H), 4.39 (dd, 1H, *J* = 9.1, 9.1 Hz), 4.22 (dd, 1H, *J* = 7.8, 13.8 Hz), 4.17 (dd, 1H, *J* = 6.0, 6.0 Hz), 3.90 (brdd, 1H, *J* = 5.6, 6.0 Hz), 3.83 (m, 1H), 3.77 (m, 1H), 3.64 (m, 1H), 3.62 (m, 2H), 3.58 (brd, 1H, *J* = 5.3 Hz), 3.52 (brdd, 1H, *J* = 5.3, 13.8 Hz), 3.50 (m, 1H), 3.47 (m, 1H), 2.93 (dd, 1H, *J* = 4.4, 13.5 Hz), 2.80 (dd, 1H, *J* = 6.7, 9.1 Hz), 2.64 (dd, 1H, *J* = 10.6, 13.5 Hz), 0.72 (d, 3H, *J* = 6.7 Hz); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 171.0, 170.9, 170.1, 169.9, 169.3, 168.1, 159.3 $\times$ 2, 156.0, 143.2, 130.4 $\times$ 2, 128.1 $\times$ 2, 127.7, 126.4, 115.0 $\times$ 2, 70.7, 70.4, 60.8, 57.4, 56.7, 56.3, 56.2, 54.4 $\times$ 2, 53.5, 43.6, 42.8, 42.2, 41.0, 35.8, 19.1; HRMS (ESI-TOF) calcd for C₃₆H₄₉N₁₂O₁₀ [M+H]⁺ 809.3694 found 809.3701.

Boc-Gly-(2*S*,3*R*)-β-MePhe-D-Tyr(Bn)-OMe (29**):** To a solution of Boc-(2*S*,3*R*)-β-MePhe-OH² (150 mg, 0.537 mmol, 1.00 equiv.) in CH₂Cl₂ (5.40 mL) was added H-D-Tyr(Bn)-OMe (HCl salt) (190 mg, 0.591 mmol, 1.10 equiv.), DIEA (0.275 mL, 1.60 mmol, 3.00 equiv.), and PyBOP (363 mg, 0.699 mmol, 1.30 equiv.) at room temperature. After being stirred at the same temperature for 12 h, the reaction mixture was poured into 3 M HCl and the aqueous phase was extracted with ethyl acetate. The combined extract was washed with H₂O and aqueous NaHCO₃ and brine, dried over Na₂SO₄, and filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue was added a solution of 4 M HCl in 1,4-dioxane (4.00 mL) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in CH₂Cl₂ (4.00 mL) was added Boc-Gly-OH (102 mg, 0.644 mmol, 1.20 equiv.), DIEA (277 μ L, 1.61 mmol, 3.00 equiv.), and PyBOP (363 mg, 0.699 mmol, 1.30 equiv.) at room temperature. After being stirred at the same temperature for 12 h, the reaction

mixture was poured into 3 M HCl and the aqueous phase was extracted with ethyl acetate. The combined extract was washed with H₂O, saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, and filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 40% ethyl acetate in hexane) to give **29** (286 mg, 0.473 μ mol, 3 steps 88%) as a white solid: mp 84-88 °C; $[\alpha]_D^{22}$ -6.18 (*c* = 1.00, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.20-7.42 (m, 10H), 6.83 (d, 1H, *J* = 9.3 Hz), 6.81 (d, 1H, *J* = 7.8 Hz), 6.69 (d, 1H, *J* = 7.8 Hz), 6.37 (brd, 1H, *J* = 7.8 Hz), 5.21 (dd, 1H, *J* = 5.3, 5.8 Hz), 5.02 (s, 2H), 4.65 (dd, 1H, *J* = 9.3, 9.3 Hz), 4.61 (dt, 1H, *J* = 6.2, 7.8 Hz), 3.81 (dd, 1H, *J* = 5.8, 17.1 Hz), 3.73 (dd, 1H, *J* = 5.8, 17.1 Hz), 3.58 (s, 3H), 3.20 (dt, 1H, *J* = 7.8, 7.8 Hz), 2.73 (dd, 1H, *J* = 6.2, 14.1 Hz), 2.55 (m, 1H), 1.46 (s, 9H), 1.29 (d, 3H, *J* = 7.8 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 171.6, 170.1, 169.6, 158.0, 142.4, 137.1, 130.2 $\times$ 2, 128.8 $\times$ 2, 128.7 $\times$ 2, 128.0, 127.9 $\times$ 3, 127.6 $\times$ 2, 127.3, 115.0 $\times$ 2, 80.5, 70.1, 58.7, 53.3, 52.2, 44.5, 42.6, 37.1, 28.4, 17.1; FT-IR (neat) 3411, 3300, 3017, 2400, 1735, 1654, 1510, 1214, 752 cm⁻¹; HRMS (ESI-TOF) calcd for C₃₄H₄₂N₃O₇ [M+H]⁺ 604.3022 found 604.3004.

Boc-Gly-(2*S*,3*R*)- β -MePhe-D-Tyr(Bn)-Aiha-A-Aiha-B-Ser(Bn)-OAllyl **30:** To a solution of **22** (10.0 mg, 8.25 μ mol, 1.00 equiv.) was added a solution of 4 M HCl in 1,4-dioxane (1.00 mL) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue of **S13** was used for the next reaction without further purification.

To a solution of Boc-Gly-(2*S*,3*R*)- β -MePhe-D-Tyr(Bn)-OMe (**29**) (7.6 mg, 12 μ mol, 1.5 equiv.) in THF (673 μ L) and H₂O (135 μ L) was added LiOH·H₂O (1.6 mg, 41 μ mol, 4.9 equiv.) at room temperature. After being stirred at the same temperature for 2 h, the reaction mixture was poured into ice-cooled 1 M HCl and the aqueous phase was extracted with CHCl₃. The combined extract was dried over Na₂SO₄, and filtered and concentrated *in vacuo*. The residue of **S14** was used for the next reaction without further purification.

To a solution of the residue of **S13** in CH₂Cl₂ (0.250 mL) was added the residue of **S14**, DIEA (5.7 μ L, 33 μ mol, 4.0 equiv.), and HATU (9.4 mg, 25 μ mol, 3.0 equiv.) at room temperature under argon. After being stirred at the same temperature for 12 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 4% MeOH in CHCl₃) to give **30** (12.2 mg, 7.30 μ mol, 2 steps 88%) as a white solid: HRMS (ESI-TOF) calcd for C₉₀H₉₉N₁₂O₂₁ [M+H]⁺ 1683.7048, found 1683.7095. The solubility of the obtained hexapeptide **30** against various solvents was insufficient for NMR analysis. Therefore, protecting groups were removed for structural confirmation of the hexapeptide as shown below.

Boc-Gly-(2S,3R)-β-MePhe-D-Tyr-Aiha-A-Aiha-B-Ser-OH (S15) (TFA salt): To a solution of **30** (6.0 mg, 3.8 μ mol, 1.0 equiv.) in 1,4-dioxane/H₂O = 3/1 (1.00 mL) was added Pd(PPh₃)₄ (0.6 mg, 0.5 μ mol, 13 mol%) and DMBA (0.6 mg, 4 μ mol, 1 equiv.) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in MeOH (0.50 mL), THF (0.50 mL), H₂O (130 μ L) and HCO₂H (63 μ L) was added Pd(OH)₂ (20% on carbon, 12.0 mg) at room temperature under argon. After being stirred at the same temperature for 24 h under H₂ gas atmosphere, the reaction mixture was filtered and concentrated *in vacuo*. The residue was purified by reversed phase column chromatography (Bond Elut-C18: 0% to 40% MeCN in H₂O, 0.02% TFA) to give **S15** (TFA salt) (3.1 mg, 3.0 μ mol, 2 steps 78%) as a white solid: [α]_D²³ +11.4 (c = 0.10, H₂O/MeOH = 1/1); ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.16 (brs, 1H), 8.34 (d, 1H, *J* = 8.4 Hz), 8.32 (d, 1H, *J* = 8.4 Hz), 8.29 (d, 1H, *J* = 7.2 Hz), 8.26 (d, 1H, *J* = 9.0 Hz), 8.00 (brs, 1H), 7.88 (brs, 1H), 7.82 (brs, 1H), 7.74 (brs, 1H), 7.69 (d, 1H, *J* = 8.4 Hz), 7.64 (brs, 1H), 7.54 (brs, 1H), 7.18 (dd, 1H, *J* = 7.2, 7.2 Hz), 7.15 (d, 1H, *J* = 7.2 Hz), 7.12 (t, 1H, *J* = 7.2 Hz), 6.96 (d, 2H, *J* = 8.4 Hz), 6.93 (t, 1H, *J* = 5.4 Hz), 6.60 (d, 1H, *J* = 8.4 Hz), 5.61 (brd, 1H, *J* = 4.2 Hz), 5.45 (brs, 1H), 4.56 (m, 2H), 4.48 (m, 1H), 4.37 (dd, 1H, *J* = 8.4, 8.4 Hz), 4.31 (m, 1H), 3.92 (brs, 1H), 3.83 (m, 1H), 3.70 (m, 1H), 3.63 (m, 4H), 3.54 (m, 1H), 3.52 (m, 1H), 3.45 (dd, 1H, *J* = 5.4, 16.2 Hz), 2.94 (m, 1H), 2.62 (dd, 1H, *J* = 8.4, 14.4 Hz), 2.38 (m, 1H), 1.36 (s, 9H), 0.88 (d, 1H, *J* = 6.6 Hz); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 172.1, 171.4, 170.4, 170.2, 169.2, 169.0, 159.4, 159.2, 156.1, 156.0, 143.0, 130.2, 128.0 $\times$ 2, 127.6, 126.4, 115.0, 78.3, 72.0,

70.8, 61.5, 57.1, 56.3 $\times$ 2, 55.0, 54.6, 54.2 $\times$ 2, 44.7, 43.3, 42.9, 42.0, 37.4, 28.4, 15.6; HRMS (ESI-TOF) calcd for C₄₁H₅₉N₁₂O₁₃ [M+H]⁺ 927.4324 found 927.4346.

Macrolactam 31: To a solution of **30** (6.0 mg, 3.6 μ mol, 1.0 equiv.) in 1,4-dioxane/H₂O = 3/1 (1.00 mL) was added Pd(PPh₃)₄ (0.6 mg, 0.5 μ mol, 14 mol%) and DMBA (0.6 mg, 4 μ mol, 1 equiv.) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by short-pass column chromatography on silica-gel (0% to 20% MeOH in CHCl₃). The obtained residue was used for the next reaction without further purification.

To a solution of the residue in CH₂Cl₂ (0.500 mL) was added TFA (25.0 μ L) at room temperature under argon. After being stirred at the same temperature for 30 min, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in CH₂Cl₂ (3.6 mL) was added DIEA (2.5 μ L, 14 μ mol, 4.0 equiv.) and HATU (6.8 mg, 18 μ mol, 5.0 equiv.) at room temperature under argon. After being stirred at the same temperature for 2 days, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 5% MeOH in CHCl₃) to give **31** (2.8 mg, 1.8 μ mol, 3 steps 51%) as a white solid: HRMS (ESI-TOF) calcd for C₈₂H₈₅N₁₂O₁₈ [M+H]⁺ 1525.6105 found 1525.6114. The solubility of the obtained macrolactam **31** against various solvents was insufficient for NMR analysis.

Mannopeptimycin aglycone (32) (TFA salt): To a solution of **31** (4.0 mg, 2.6 μ mol, 1.0 equiv.) in

MeOH (0.500 mL), THF (0.500 mL), H₂O (130 μ L) and HCO₂H (63 μ L) was added Pd(OH)₂ (20% on carbon, 12.0 mg) at room temperature under argon. After being stirred at the same temperature for 24 h under H₂ gas atmosphere, then the reaction mixture was filtered and concentrated *in vacuo*. The residue was purified by reversed phase column chromatography (Bond Elut-C18: 0% to 30% MeCN in H₂O, 0.02% TFA) to give **32** (TFA salt) (2.0 mg, 2.2 μ mol, 84%) as a white solid; $[\alpha]_D^{24}$ -3.9 (c = 0.05, H₂O/MeOH = 1/1); ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.18 (brs, 1H), 8.43 (d, 1H, *J* = 6.6 Hz), 8.18 (brs, 1H), 8.12 (d, 1H, *J* = 8.4 Hz), 8.01 (brs, 1H), 7.97 (brs, 1H), 7.93 (brs, 1H), 7.60-7.82 (m, 5H), 7.77-7.80 (m, 1H), 7.22 (m, 2H), 7.19 (d, 2H, *J* = 6.6 Hz), 7.13-7.18 (m, 1H), 6.82 (d, 2H, *J* = 7.8 Hz), 6.58 (d, 2H, *J* = 7.8 Hz), 5.62 (d, 1H, *J* = 5.4 Hz), 5.39 (brs, 1H), 5.13 (brs, 1H), 4.48 (dd, 1H, *J* = 7.2, 7.8 Hz), 4.37 (dd, 1H, 8.4, 8.4 Hz), 4.30 (m, 2H), 4.24 (brs, 1H), 3.94 (brs, 1H), 3.84 (m, 1H), 3.67-3.74 (m, 1H), 3.62-3.67 (m, 2H), 3.61-3.65 (m, 1H), 3.56-3.63 (m, 1H), 3.54-3.59 (m, 1H), 3.52-3.56 (m, 1H), 3.37-3.44 (m, 1H), 3.18-3.23 (m, 1H), 3.15-3.19 (m, 1H), 2.64 (m, 1H), 2.41 (m, 1H), 1.07 (d, 3H, *J* = 7.2 Hz); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 170.6, 170.4, 170.1, 169.8, 169.3, 168.7, 159.3 $\times$ 2, 156.1, 143.2, 130.2, 128.2, 127.8, 127.5, 126.5, 114.9, 70.7, 70.0, 61.2, 57.6, 56.8, 56.0, 55.9, 54.6, 54.2, 53.8, 44.2, 43.0, 42.4, 40.2, 36.3, 16.6; HRMS (ESI-TOF) calcd for C₃₆H₄₉N₁₂O₁₀ [M+H]⁺ 809.3694 found 809.3692.

Marfey's analysis of Boc-Gly-(2S,3S)-β-MePhe-D-Tyr(Bn)-OMe (23b): To a solution of Boc-Gly-(2S,3S)-β-MePhe-D-Tyr(Bn)-OMe (**23b**) (1.0 mg) in MeOH (0.25 mL) was added Pd(OH)₂ (20% on carbon, 0.2 mg) at room temperature under argon. After being stirred at the same temperature for 8 h under H₂ gas atmosphere, to the reaction mixture was added THF (200 μ L), H₂O (40.0 μ L) and LiOH·H₂O (0.3 mg) at room temperature under argon. After being stirred at the same temperature for 2 h, the reaction mixture was poured into ice-cooled 1 M HCl and the aqueous phase was extracted with CHCl₃. The combined extract was dried over Na₂SO₄, and filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

The residue was dissolved in degassed 6 M HCl (0.5 mL) in a sealed vial and heated at 100 °C for 16 h. The solvent and the acid were removed *in vacuo* and the residue was dissolved in H₂O (0.1 mL). The solution was then treated with 6.0% NEt₃ (30 μ L) and 1.0% 1-fluoro-2, 4-dinitrophenyl-5-L-alanineamide (FDAA) in acetone (40 μ L) at 40 °C for 1 h. The reaction mixture was diluted with MeCN/H₂O = 1/1 (0.2 mL) and the resultant solution was analyzed by HPLC (see below). The mobile phase was monitored by both UV absorption and negative ESIMS.

HPLC analysis: Inertsil® ODS-3 column: 4.6 $\times$ 250 mm, 3 μ m, linear gradient solvent: 10-100%

MeCN/H₂O with 0.1% formic acid over 30 min, flow rate: 1.0 mL/min. The retention times and the negative ion, [M-H]⁻, of the obtained FDAA derivatives of the (2*S*,3*S*)-β-MePhe, and standard sample are given in parentheses: obtained (2*S*,3*S*)-β-MePhe (20.7 min, m/z 430), standard sample (2*S*,3*S*)-β-MePhe (20.7 min, m/z 430). During a comparison of the above retention times, no epimerization of the (2*S*,3*S*)-β-MePhe residue was observed.

Marfey's analysis of Boc-Gly-(2*S*,3*S*)-β-MePhe-D-Tyr(Bn)-Aiha-A-Aiha-B-Ser(Bn)-OAllyl **26:**

To a solution of Boc-Gly-(2*S*,3*S*)-β-MePhe-D-Tyr(Bn)-Aiha-A-Aiha-B-Ser(Bn)-OAllyl **26** (1.0 mg) in 1,4-dioxane/H₂O = 3/1 (200 μL) was added Pd(PPh₃)₄ (0.1 mg) and DMBA (0.1 mg) at room temperature under argon. After being stirred at the same temperature for 1 h, to the reaction mixture was added MeOH (0.400 mL), THF (0.400 mL), H₂O (0.100 mL), HCO₂H (50.0 μL) and Pd(OH)₂ (20% on carbon, 2.0 mg) at room temperature under argon. After being stirred at the same temperature for 24 h under H₂ gas atmosphere, the reaction mixture was filtered and concentrated *in vacuo*. The residue was purified by reversed phase column chromatography (Bond Elut-C18: 0% to 40% MeOH in H₂O, 0.02% TFA).

The eluted compound was dissolved in degassed 6 M HCl (0.5 mL) in a sealed vial and heated at 100 °C for 16 h. The solvent and the acid were removed *in vacuo* and the residue was dissolved in H₂O (0.1 mL). The solution was then treated with 6.0% NEt₃ (30 μL) and 1.0% FDAA in acetone (40 μL) at 40 °C for 1 h. The reaction mixture was diluted with MeCN/H₂O = 1/1 (0.2 mL) and the resultant solution was analyzed by HPLC (see below). The mobile phase was monitored by both UV absorption and negative ESIMS.

HPLC analysis: Inertsil® ODS-3 column: 4.6 × 250 mm, 3 μm, linear gradient solvent: 10-100% MeCN/H₂O with 0.1% formic acid over 30 min, flow rate: 1.0 mL/min. The retention times and the negative ion, [M-H]⁻, of the obtained FDAA derivative of the D-Tyr and standard samples are given in parentheses: obtained D-Tyr (12.1 min, m/z 432; 17.2 min, m/z 684), standard sample D-Tyr (12.2 min, m/z 432; 17.4 min, m/z 684), standard sample L-Tyr (11.9 min, m/z 432; 16.6 min, m/z 684). During a comparison of the above retention times, no epimerization of the D-Tyr residue was observed.

Marfey's analysis of proposed mannopeptimycin aglycone (6) (TFA salt): Proposed mannopeptimycin aglycone (**6**) (0.3 mg) was dissolved in 6 M degassed HCl (0.5 mL) in a sealed vial and heated at 100 °C for 16 h. The solvent and the acid were removed *in vacuo* and the residue was dissolved in H₂O (0.1 mL). The solution was then treated with 6.0% NEt₃ (30 µL) and 1.0% FDAA in acetone (40 µL) at 40 °C for 1 h. The reaction mixture was diluted with MeCN/H₂O = 1/1 (0.2 mL) and the resultant solution was analyzed by HPLC (see below). The mobile phase was monitored by both UV absorption and negative ESIMS.

HPLC analysis: Inertsil® ODS-3 column: 4.6 × 250 mm, 3 µm, linear gradient solvent: 30-70% MeCN/H₂O with 0.1% formic acid over 30 min, flow rate: 1.0 mL/min. The retention times and the negative ion, [M-H]⁻, of the obtained FDAA derivative of the L-Ser and standard samples are given in parentheses: obtained L-Ser (8.6 min, m/z 356), standard sample L-Ser (8.6 min, m/z 356), standard sample D-Ser (8.8 min, m/z 356). During a comparison of the above retention times, no epimerization of the L-Ser residue was observed.

The assignment of the signals in the observed NMR spectra for dipeptide **S6** was difficult because of the presence of rotamers. Therefore, oxazolidine **S6** was converted into amino alcohol **S16** to enable NMR analysis and confirmation of epimerization as shown below.

Preparation of Boc-Aiha-B-Ser(Bn)-OAllyl S16 for confirmation of epimerization: To a solution of Boc-Aiha-B-Ser(Bn)-OAllyl **S6** (9.0 mg, 11 µmol, 1.0 equiv.) was added a solution of 4 M HCl in 1,4-dioxane (1.5 mL) and H₂O (15 µL) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in CH₂Cl₂ (0.500 mL) was added Boc₂O (4.1 mg, 17 µmol, 1.5 equiv.)

and K_2CO_3 (4.6 mg, 33 μmol , 3.0 equiv.) at room temperature. After being stirred at the same temperature for 12 h, the reaction mixture was poured into 2 M aqueous NaHSO_4 and the aqueous phase was extracted with ethyl acetate. The combined extract was washed with brine, dried over Na_2SO_4 , and filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 20% acetone in toluene) to give amino alcohol **S16** (6.9 mg, 8.9 μmol , 2 steps 82%) as a colorless oil: $[\alpha]_{\text{D}}^{20}$ -38.9 ($c = 0.45$, CHCl_3); ^1H NMR (400 MHz, acetone- d_6) δ 9.13 (brs, 1H), 8.08 (brs, 1H), 7.22-7.57 (m, 15H), 6.07 (brs, 1H), 5.90 (ddt, 1H, $J = 5.3, 10.8, 17.6$ Hz), 5.33 (dd, 1H, $J = 3.4, 17.6$ Hz), 5.25 (s, 2H), 5.19 (dd, 1H, $J = 2.9, 10.8$ Hz), 5.12 (s, 2H), 4.74 (m, 1H), 4.62 (m, 2H), 4.55 (m, 1H), 4.49 (d, 2H, $J = 3.4$ Hz), 4.19 (brs, 1H), 4.03 (brs, 1H), 3.99 (m, 1H), 3.92 (dd, 1H, $J = 3.9, 9.8$ Hz), 3.87 (brs, 1H), 3.74 (dd, 1H, $J = 3.7, 9.8$ Hz), 1.43 (s, 9H); ^{13}C NMR (100 MHz, acetone- d_6) δ 170.2 $\times$ 2, 156.9, 151.7, 138.9, 137.0, 133.1 $\times$ 2, 129.1 $\times$ 2, 129.0 $\times$ 4, 128.7 $\times$ 2, 128.6, 128.4 $\times$ 2, 128.3 $\times$ 4, 118.1, 80.2, 73.6, 70.4, 68.2, 67.3, 66.2 $\times$ 2, 56.2, 54.2, 53.9, 47.1, 28.3; FT-IR (neat) 3330, 2978, 1759, 1707, 1663, 1598, 1498, 1454, 1368, 1310, 1250, 1165, 1060, 1026, 768, 744, 698 cm^{-1} ; HRMS (ESI-TOF) calcd for $\text{C}_{40}\text{H}_{48}\text{N}_5\text{O}_{11}$ $[\text{M}+\text{H}]^+$ 774.3359 found 774.3364.

Preparation of Boc-Aiha-A-Ser(Bn)-OAllyl **S17 (epimer) for confirmation of epimerization:** To a solution of **S7** (60.0 mg, 0.105 mmol, 1.00 equiv.) in THF (4.30 mL) and H_2O (0.859 mL) was added $\text{LiOH}\cdot\text{H}_2\text{O}$ (23.7 mg, 0.515 mmol, 4.90 equiv.) at 0 $^\circ\text{C}$. After being stirred at the same temperature for 10 min, the reaction mixture was poured into ice-cooled 1 M HCl and the aqueous phase was extracted with CHCl_3 . The combined extract was dried over Na_2SO_4 , and filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in CH_2Cl_2 (3.80 mL) was added H-Ser(Bn)-OAllyl (**19**) (HCl salt) (34.3 mg, 0.126 mmol, 1.20 equiv.), DIEA (54.4 μL , 0.315 mmol, 3.00 equiv.), and HATU (55.9 mg, 0.147 mmol, 1.40 equiv.) at room temperature under argon. After being stirred at the same temperature for 12 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica-gel (0% to 5% MeOH in CHCl_3) to give Boc-Aiha-A-Ser(Bn)-OAllyl **S17** (64.9 mg, 0.0840 mmol, 2 steps 80%) as a colorless oil: $[\alpha]_{\text{D}}^{23}$ +16.6 ($c = 1.00$, CHCl_3); ^1H NMR (400 MHz, acetone- d_6) δ 8.84 (brs, 1H), 8.00 (brs, 1H), 7.12-7.55 (m, 15H), 6.12 (brs, 1H), 5.90 (ddt, 1H, $J = 5.4, 10.8, 17.2$ Hz), 5.33 (dd, 1H, $J = 3.4, 17.2$ Hz), 5.25 (s, 2H), 5.18 (dd, 1H, $J = 3.4, 10.8$ Hz), 5.10 (s, 2H), 4.76 (m, 1H), 4.62 (m, 2H), 4.55 (d, 2H, $J =$

12.2 Hz), 4.49 (d, 2H, $J = 12.2$ Hz), 3.95 (brs, 1H), 3.95 (dd, 1H, $J = 3.4, 9.8$ Hz), 3.76 (dd, 1H, $J = 3.4, 9.8$ Hz), 1.41 (s, 9H); ^{13}C NMR (100 MHz, acetone- d_6) δ 170.9, 170.6, 156.2, 151.8 $\times$ 2, 151.6, 138.9, 137.0, 133.0 $\times$ 2, 129.1 $\times$ 2, 129.0 $\times$ 6, 128.7 $\times$ 2, 128.6, 128.4 $\times$ 2, 128.3 $\times$ 2, 118.2, 79.9, 73.5, 70.3, 68.2, 67.4, 66.3, 66.1, 56.4, 54.2, 53.9, 45.7, 28.4; FT-IR (neat) 3334, 3032, 2978, 1749, 1713, 1661, 1599, 1522, 1498, 1454, 1382, 1368, 1308, 1255, 1168, 1049, 1025, 769, 744, 698, 609 cm^{-1} ; HRMS (ESI-TOF) calcd for $\text{C}_{40}\text{H}_{48}\text{N}_5\text{O}_{11}$ $[\text{M}+\text{H}]^+$ 774.3359 found 774.3365. From the comparison of NMR spectra of **S16** and **S17**, no epimerization of the Aiha-B residue was observed.

Preparation of Boc-Aiha-B-Aiha-B-Ser(Bn)-OAllyl **S18 (epimer) for confirmation of epimerization:** To **18** (30.0 mg, 49.2 μmol , 1.00 equiv.) was added a solution of 4 M HCl in 1,4-dioxane (2.00 mL) and H_2O (20 μL) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in THF (1.00 mL) and H_2O (0.500 mL) was added Boc_2O (61.9 mg, 0.246 mmol, 5.00 equiv.) and K_2CO_3 (20.4 mg, 0.148 mmol, 3.00 equiv.) at room temperature. After being stirred at the same temperature for 12 h, the reaction mixture was poured into 2 M aqueous NaHSO_4 and the aqueous phase was extracted with ethyl acetate. The combined extract was washed with brine, dried over Na_2SO_4 , and filtered, and concentrated *in vacuo*. The residue was purified by short-pad column chromatography on silica-gel (0% to 20% acetone in toluene) to give crude amino alcohol **S19** (20.8 mg). The compound was used for the next reaction without further purification.

To a solution of methyl ester **S19** (10.0 mg, 17.0 μmol , 1.00 equiv.) in THF (720 μL) and H_2O (120 μL) was added $\text{LiOH}\cdot\text{H}_2\text{O}$ (4.0 mg, 86 μmmol , 4.9 equiv.) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was poured into ice-cooled 1 M HCl and the aqueous phase was extracted with CHCl_3 . The combined extract was dried over Na_2SO_4 , and filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in CH_2Cl_2 (0.300 mL) was added H-Aiha-B-Ser(Bn)-OAllyl **20** (HCl salt) (12.6 mg, 18.7 μmol , 1.10 equiv.), DIEA (11 μL , 51 μmol , 3.0 equiv.), and HATU (9.7 mg, 26 μmol , 1.5 equiv.) at room temperature under argon. After being stirred at the same temperature for 12 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by chromatography on silica-gel (0% to 5% MeOH in CHCl_3) to give Boc-Aiha-B-Aiha-B-Ser(Bn)-OAllyl **S18** (8.5 mg, 7.0 μmol , 4 steps 30%) as a colorless oil. HRMS (ESI-TOF) calcd for $\text{C}_{62}\text{H}_{70}\text{N}_9\text{O}_{17}$ $[\text{M}+\text{H}]^+$

1212.4890 found 1212.4882. The solubility of the obtained tripeptide **S18** against various solvents was insufficient for NMR analysis.

HPLC analysis: Inertsil® ODS-3 column: 4.6 × 250 mm, 3 µm, linear gradient solvent: 10-100% MeCN/H₂O with 0.1% formic acid over 15 min, flow rate: 1.0 mL/min. The retention time of **S18**: 9.00 min. During a comparison of retention times for the desire tripeptide **22** (9.13 min) and tripeptide epimer **S18** (9.00 min), no epimerization of the AihA-A residue was observed.

Antimicrobial Assay: Antimicrobial activity was measured by the conventional paper disk diffusion method.^{3,4} In this method, each paper disk (φ 6 mm, Whatman) containing the 2.5, 5, and 10 µg of the compounds **S8**, **S12**, **S15**, **6**, and **32**, and 0.15, 0.31, 0.63, 1.25, and 2.5 µg of erythromycin and vancomycin as positive controls were placed directly onto an LB agar plate including 0.2% of the liquid culture of the *S. aureus* IAM1011. The plate was then incubated at 28 °C for 24 h. The activity was estimated by measuring the diameters of the inhibition zones as shown in Figure S1. The positive controls, erythromycin and vancomycin formed inhibition zones, diameter = 21, 14 mm, respectively, at 2.5 µg/disc. On the other hand, no inhibition zones were observed in the case of compounds, **S8**, **S12**, **S15**, **6**, and **32**.

Figure S1. Agar diffusion method used to test antimicrobial activity against of *S aureus*. IAM1011 of (A) erythromycin and vancomycin as positive controls, and (B) compounds **S8**, **S12**, **S15**, **6**, and **32**. The development of a clear zone around the disc was indicative of the ability of the antimicrobial compounds to kill bacteria.

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NMR spectra

Alcohol 7

¹H NMR

¹³C NMR

¹H NMR ¹³C NMR

Oxazolidine 12

¹H NMR

¹³C NMR

Carbamate 13

¹H NMR

¹³C NMR

Oxazolidine S1

¹H NMR

¹³C NMR

Guanidine S2

¹H NMR

¹³C NMR

Protected Aiha-A 15

¹H NMR

¹³C NMR

Amino alcohol 16

¹H NMR

¹³C NMR

Oxazolidine S3

¹H NMR

¹³C NMR

Protected Aiha-B 18

¹H NMR

¹³C NMR

Aiha-A S4 (TFA salt)

¹H NMR

Aiha-B S5 (TFA salt)

¹H NMR

Amino alcohol S7

¹H NMR

¹³C NMR

Boc-Aiha-A-Aiha-B-Ser-OH (S8) (TFA salt)

¹H NMR

¹³C NMR

¹H NMR

Alloc-Gly-(2*S*,3*S*)- β -MePhe-D-Tyr(Bn)-OMe (23a)

^1H NMR

^{13}C NMR

Boc-Gly-(2*S*,3*S*)- β -MePhe-D-Tyr(Bn)-OMe (23b)

^1H NMR

^{13}C NMR

Boc-Gly-(2S,3S)- β -MePhe-D-Tyr-Aiha-A-Aiha-B-Ser-OH (S12) (TFA salt)

^1H NMR

^{13}C NMR

Proposed mannopeptimycin aglycone (6) (TFA salt)

¹H NMR

¹³C NMR

^1H - ^1H COSY

HMBC

HSQC

Mannopectimycin aglycone (6) (TFA salt) obtained by degradation of naturally occurring mannopectimycin β .

^1H NMR

HSQC

Boc-Gly-(2*S*,3*R*)- β -MePhe-D-Tyr(Bn)-OMe (29)

^1H NMR

^{13}C NMR

¹H NMR

Mannopectimycin aglycone (32) (TFA salt)

¹H NMR

¹³C NMR

^1H - ^1H COSY

HMBC

HSQC

Boc-Aiha-B-Ser(Bn)-OAllyl S16

¹H NMR

Boc-Aiha-A-Ser(Bn)-OAllyl S17

¹H NMR

¹³C NMR